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**Contributions to Analytical Chemistry of Vitamin B₁₂**

**Determination of Small Amounts of Cyanocobalamine in Presence of Cyanide and Analogues of Vitamin B₁₂ by Means of a Cyanide Electrode without Distillation of Hydrogen Cyanide**

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With 2 Figures

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The methods of determining Vitamin B₁₂ can be divided into two main classes, biological and chemical. Although precise, the biological methods are slow and complicated. The chemical methods, which have been reviewed¹, are divided into direct and indirect methods, the former using optical, electrochemical and radiometric techniques, etc., and the latter being based on determination of characteristic groups in the Vitamin B₁₂ molecule, such as PO₄³⁻, Co³⁺, CN⁻, 5,6-dimethylbenzimidazole.

One indirect method² is based on converting the phosphate content into phosphomolybdate and determining the molybdenum in this colorimetrically or polarographically. A voltammetric determination of the cobalt in Vitamin B₁₂ and its analogues, without prior decomposition, has been devised³, and a kinetic method⁴ and an infrared absorption method⁵ for the cyanide group. The cyanide has also been determined colorimetrically with pyridine-pyrazolone reagent after photolysis by ultraviolet irradiation in tartaric acid

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medium\textsuperscript{6}, 1 \mu g of Vitamin B\textsubscript{12} (0.02 \mu g of CN\textsuperscript{−}) being determined with an error of 13\%\textsubscript{o}, and 5 \mu g with an error of 5\%\textsubscript{o}. On the basis of a study of the equilibria between cyanide, and Vitamins B\textsubscript{12}, B\textsubscript{12a}, and B\textsubscript{12b}, for which apparent equilibrium constants were measured\textsuperscript{7}, a method was developed for determining these three vitamins in presence of each other by measuring the cyanide in cyanocobalamine before and after quantitative conversion of the analogues into cyanocobalamine.

In other works\textsuperscript{8-11}, hydrocyanic acid liberated by irradiation of Vitamin B\textsubscript{12} is converted into sodium cyanide and measured colorimetrically with benzidine or guaiac resin. Alternatively, Vitamin B\textsubscript{12} is treated with 60\%\textsubscript{o} sulphuric acid, the hydrocyanic acid formed is neutralized, treated with chloramine-T, and determined with pyrazolone reagent in presence of a phosphate buffer, the method being automated\textsuperscript{12}.

The cyanide liberated by reduction or irradiation has also been determined by means of an ion-selective electrode for cyanide, after distillation of the hydrocyanic acid and its conversion into sodium cyanide\textsuperscript{13}, the error being \( \pm 5\%\textsubscript{o}\).

In the present work, based on the equilibria between various forms of Vitamin B\textsubscript{12} and cyanide at pH \( \geq 10 \), we concluded that the distillation step was unnecessary.

The system

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\text{B}_{12}-\text{CN} \xrightarrow{\text{UV, H}^+ (pH 3-4)} \text{B}_{12}-\text{OH}^+ + \text{HCN} \xrightarrow{\text{OH}^- (pH 12-13)} \text{B}_{12}-\text{OH} + \text{CN}^- \]

was investigated earlier and the equilibria compared with those which take place starting from B\textsubscript{12}-OH and NaCN or KCN, under the same conditions. Special attention was paid to thermolysis and photolysis of cyanocobalamine and cobinamide (Factor B) followed by cyanide determination with a cyanide-selective electrode, as a means of estimating the stability of the Co—CN bond.

**Experimental**

**Apparatus**

A Radelkis cyanide electrode (Model OP-CN-7111, Radelkis, Budapest), a saturated calomel electrode, an agar-agar bridge, and a scale-expansion pH-meter (Pye, Model 78) were used for potentiometry.

The 25-ml volumetric flasks used for sample irradiation were made of ordinary glass, the absorption spectrum of which is shown in Fig. 1.

Before measurements the cyanide electrode was kept for 5 hr. in 0.1 M potassium nitrate buffer solution at pH 11 (adjusted with sodium hydroxide), then was carefully washed with distilled water and standardized.